Oviposition response and development of the egg-pupal parasitoid *Fopius arisanus* on *Bactrocera oleae*, a tephritid fruit fly pest of olive in the Mediterranean basin

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Abstract

To date, information is wanting with regard to the use of new exotic parasitoids against olive fruit fly, *Bactrocera* (=*Dacus*) *oleae* (Gmelin) (Diptera: Tephritidae), a serious pest of olives *Olea europaea* L., in the Mediterranean basin. We investigated the oviposition response and developmental biology on *B. oleae* of *Fopius* (=*Biosteres*) *arisanus* (Sonan) (=*Opius oophilus* Fullaway) (Hymenoptera: Braconidae), an egg-pupal parasitoid of tephritid fruit flies, never tested before as a potential parasitoid of this host. Our results showed that olive fruits infested with *B. oleae* eggs exerted a relevant attraction to gravid *F. arisanus* and represented a stimulus for oviposition. Nevertheless they were not as attractive to female parasitoids as the Mediterranean fruit fly, *Ceratitis capitata* Wiedemann (Diptera: Tephritidae), eggs infested papaya fruits (*Carica papaya* L.). In our experimental conditions, *F. arisanus* completed development in *B. oleae* within 33 ± 1.7 days (males) and 35 ± 1.6 (females). Increases in host egg to female parasitoid ratios of 1:1, 5:1, 10:1 and 20:1 corresponded with decreases in the percentage of *B. oleae* parasitisation and host killing but corresponded also with increases in absolute parasitisation. Our findings are discussed in light of possibilities of utilising *F. arisanus* for biological control of olive fruit fly.

Introduction

Olives are extensively grown along the Mediterranean region. However, persistent infestation by the olive fruit fly, *Bactrocera oleae* (Gmelin), causes severe damage to olive fruits, thus affecting the quality of the produced oil and the crop's export market potential (Fimiani, 1989).

The conventional method of olive fruit fly control using chemical pesticides, although effective, has proven to be unsafe and ecologically disruptive (Cirio, 1997). Not only do they pose unwarranted risks to public health but also present ecological backlashes to non-target organisms in the environment (Heim, 1984). Generally, less than 1% of the pesticide used reaches the detrimental species at which it is directed. The rest is dispersed in the environment (Cirio, 1997).

Moreover, residues of some pesticides were even found on olives and in olive oil (Leandri et al. 1993). In light of this predicament, pest management tactics, such as sterile insect release and parasitoid augmentation, offer a viable and sound alternative in tephritid fruit flies control (Knipling, 1992).

Strategies for sterile fly releases had already been developed for *C. capitata* (Cirio, 1977), but they are hardly applicable to *B. oleae* because of the lack of an effective and low cost mass rearing method for this fruit fly species (Tzanakakis, 1989). On the other hand, efforts to exploit the effectiveness of the larval parasitoid *Opius concolor* (Szépligeti) (Hymenoptera: Braconidae) for augmentative releases against *B. oleae* were not very successful in Italy, because of problems associated with insectary rearing

(Genduso et al., 1994), acclimatisation, and parasitoid behaviour (Fimiani, 1982).

Fopius arisanus (Sonan) is one of the only two known opiine egg-pupal parasitoids. It was originally collected from puparia of the oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), but it was found able to attack at least seven species of tephritid fruit flies in the Western Hemisphere (Prokopy & Webster, 1978; Wharton & Gilstrap, 1983) and thought also to parasitise 1st instar larvae. Parasitoids complete development inside the host and emerge as adults, usually a few days after emergence of fruit flies from unparisitized puparia (Haramoto, 1953; Clausen et al., 1965). The puncture by the ovipositing female parasitoid may result in unsuccessful parasitisation due to direct killing of host eggs (Newell & Rathburn, 1951; Nishida & Haramoto, 1953).

Efforts to utilise *F. arisanus* in biological control of tephritid fruit flies have been documented in Hawaii and elsewhere. Introduced from Southeast Asia in early 1940s, substantial reductions of *B. dorsalis* and *C. capitata* in the Hawaiian island chain were attributed largely to parasitisation by *F. arisanus* (Wong & Ramadan, 1987; Vargas et al., 1993). Notwithstanding, attempts to introduce *F. arisanus* to Florida from Hawaii in 1974–1975 for suppression of the Caribbean fruit fly. *Anastrepha suspensa* (Loew) (Diptera: Tephritidae), failed miserably (Baranowski et al., 1993). However, similar effort in Costa Rica was deemed successful after parasitoids were recovered from field collections of *Anastrepha* spp. puparia (Wharton et al., 1981). In Malaysia, there was evidence that *F. arisanus* development is inhibited by the presence of another parasitoid. *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae), when both occurred in multiparasitised hosts (Palacio et al., 1991).

More recently, a mass-rearing technology has been developed in Hawaii for large scale production of *F. arisanus* to suppress *B. orientalis* and *C. capitata* populations (Bautista et al., 1999). A renewed enthusiasm among pest management specialists has arisen on the potential of *F. arisanus* for augmentative biological control of fruit flies.

A culture of *F. arisanus* has been recently established in the laboratory of applied Entomology at the ENEA (National Institute for New Technologies and Environmental Safety) Research Centre in Rome (Italy). It originated from an initial stock of *F. arisanus* reared on *C. capitata*, that was developed and provided by the U.S. Pacific Basin Agricultural Research Center (USPBARC) (USDA ARS, Honolulu, Hawaii, USA), and hand carried (as parasitised puparia) via commercial flights to our laboratories. Consequently, as test parasitoids became available, we commenced a series of preliminary assays aimed at evaluating *F. arisanus* potential as biocontrol agent against key tephritid fruit fly pests in the Mediterranean basin.

To date, there is no information with regard to *B. oleae* as host of *F. arisanus* and the potential efficacy of this parasitoid against olive fruit fly. Therefore, laboratory assays were undertaken in order to (1) evaluate the host seeking and oviposition response of female parasitoids toward *B. oleae* eggs presented in olive fruits, (2) evaluate the physiological suitability of the host for the complete development of normal parasitoid progeny, (3) determine the duration of preimago development and pattern of parasitoid and host fly emergence in the new host, and (4) quantify the effect of host eggs number to female parasitoid ratio on the functional response of the parasitoid.

Materials and methods

Parasitoids and host flies. Parasitoids were allowed to emerge and reared inside plastic screen cages (30 × 30 × 30 cm). They were provided with food by streaking honey on the screen of the cage. Water was accessed inside the cage from a piece of moistened foam that was inserted into a 30-ml plastic water reservoir. Subsequently, mature females were egged and propagated on medfly, using an artificial method developed in our laboratories.

Our artificial oviposition units were made up of a plastic bottle and a thin sponge fixed tightly to the inner surface of the bottle. The bottle was perforated (holes 3 mm in diameter) to allow the placement, using a Pasteur pipette, of host eggs, previously dispersed in a water solution (about 20 000 eggs in 30 ml of water) directly onto the sponge layer. The sponge, wet slightly when the procedure of filling with *C. capitata* eggs began, adsorbed water and kept the eggs moist during the exposure to parasitoids. After the exposure period, eggs were removed from the bottle by shaking it in a water container, then sieved and finally transferred into a carrot-based larval medium (Mitchell et al., 1965). Pupation took place inside containers provided with sand. After a period of adjustment to the new rearing conditions, starting from

the 10th generation we began to produce parasitoids in sufficient numbers for laboratory assays.

Insectary-raised *C. capitata* was obtained from our fruit fly rearing facility while a colony of *B. oleae* was established in the insectary by gathering infested olives (cv. 'Itrana') from commercial orchards in Central Italy. After oviposition, olives were processed according to Tzanakakis (1989) until recovery of olive fruit fly puparia. Olive fruit flies were allowed to emerge and maintained in the same type of rearing cages used for the parasitoids, Protein yeast hydrolyzate (25%) and sucrose (75%) food mix and water were provided until females were ready to oviposit. Parasitoids and fruit flies were reared in the laboratory at temperatures of 20–24 °C, 60–79% r.h., and L14:D10 photoperiod.

Fruit substrates. Olives were gathered from trees in the orchard while fruits were still unripe. Only not punctured fruits were used in the tests. Papaya fruits, on the other hand, were purchased directly from local fruit stores.

Fruits were naturally infested by exposing olives or papayas to gravid *B. oleae* or *C. capitata*, respectively. Olives, in sets of 100 fruits each, were mounted singly into an array of holes made on a piece of styrofoam material. In the case of papaya, each of ten whole fruits was punctured with three rows of six holes (3 mm deep) in order to stimulate medfly oviposition. Fruit perforations were confined to a surface area of 12 × 5 cm. Olives and papayas (exposed singly) were exposed to *B. oleae* (100) or *C. capitata* (50), respectively, for 4 h, inside the rearing cages.

Exposure to parasitoids. In all the tests, 10–15-day old mated female parasitoids were used. In order to favour mating, 200 pairs of *F. arisanus* adults had been maintained inside rearing cages until the use in the tests. Exposure of infested fruits to parasitoids was conducted inside 30 × 30 × 30 cm rearing cages in the laboratory, with mean temperature of 24 ± 1 °C and 68 ± 5% r.h. Lighting was continuous during exposure.

Test 1. Host response of parasitoid to olive fruit fly-infested fruits. The host-seeking behaviour of female *F. arisanus* for *B. oleae* eggs was compared to that of females presented with medfly-infested papayas. Olives (with only one oviposition puncture), in set of 36 fruits, were arranged in four rows of nine each on a piece of carton box paper. The fruits, mounted on the paper using map pins, according to a technique described by Bautista & Harris (1996), approximated the size of the punctured section of the papaya (= 12 × 5 × 1 cm) that was cut out from the whole fruit after the oviposition by *C. capitata*.

Olives or papayas (in sets of two sections each) were concurrently exposed to 100 mated females of *F. arisanus* inside separate screen cages. From initial time (0 min) fruits were presented to parasitoids, and at 10-min interval, for a total exposure time of 3 h, the number of females found on fruits was counted. Data were expressed as percentage of female parasitoids assayed. In addition, those that exhibited a typical egg-laying behaviour (i.e., antennation of fruit surface, cessation of frenzied movements, bending abdomen and inserting ovipositor into fruit, pumping movements) were recorded in percentages per unit time based on number of female parasitoid found on fruits. Fresh cohorts of native *F. arisanus* females (those with no prior egg-laying experience) were used each time. Test was replicated five times.

At the end of the test, fruits were retrieved and examined under a dissecting stereoscope to determine the mean number of eggs oviposited by *C. capitata* and *B. oleae* in each puncture.

Test 2. Development and pattern of emergence of parasitoid and olive fruit fly. Preimago development of *F. arisanus* was calculated as elapsed time (in days) between initial exposure of fruit fly eggs to female parasitoids and subsequent emergence of adults from parasitised puparia.

Two hundred olive fruits, newly infested with *B. oleae* eggs (1–4 h old), were mounted on a piece of carton box (Bautista & Harris, 1996). Then, fruits were exposed to 100 *F. arisanus* females inside rearing cages for 24 h. During exposure, fluorescent lighting was continuous for purposes of maximizing parasitisation of host eggs. Subsequently, fruits were retrieved and processed according to rearing methods by Tzanakakis (1989). Rearing assay was conducted at mean temperature of 20 ± 1 °C and 65 ± 5% r.h. Within 14 ± 2 days, fruit fly puparia, which consisted of parasitised and unparasitised ones, were recovered and then placed in a plastic container where insects emerged. According to Bautista et al. (1998) fruit flies and parasitoids (males and females) were recorded as they emerged daily and results were expressed as percentage of total number of eclosed puparia.

Test 3. Effect of host egg number to female parasitoid ratio on olive fruit fly parasitisation. The propensity of *F. arisanus* to parasitise *B. oleae* was quantified by varying the amount of olive fruit fly eggs exposed to female parasitoids. The object of the test was to find out if increasing the number of olive fruit fly eggs would correspond with significantly differences in puparial recovery, percent parasitism, and increases in absolute parasitisation.

Olive fruits with only one olive fruit fly oviposition puncture were used. Thus, in order to obtain host egg to female parasitoid ratios of 1:1, 5:1, 10:1 and 20:1, the number of olives were exposed to parasitoids in groups of 10, 50, 100 and 200 fruits, respectively. Infested fruits were placed in separate rearing cages into which cohorts of ten mated female parasitoids each were introduced.

Females were allowed to parasitise host eggs for 24 h, after which fruits in each treatment were retrieved and host immatures reared until emergence of *F. arisanus*. Test was replicated ten times. For purposes of comparison, 10 sets of 50 infested olive fruits were set aside and left unexposed to parasitoids (= control treatment).

Three parameters were generated to measure the functional response of *F. arisanus* with respect to different numbers of available host eggs, namely, mean olive fruit fly puparia recovered from exposed olives, mean percent parasitism (parasitism rate), expressed as ratio between number of emerged parasitoids and total olive fruit fly puparia recovered in fruit sample, and total number of parasitoids that successfully developed from exposed olives (absolute parasitisation), calculated by adding up parasitoids obtained from each treatment replication. Olive fruit flies and parasitoids directly emerged from olives were also included in the computation.

Data analysis. In test 1, overall mean percentages of females that visited the fruit and those that responded to host eggs were compared between fruit treatments by Student *t*-test at P = 0.05. Descriptive statistics, namely, mean, standard error of the mean (S.E.) and range were generated in test 2 to indicate the duration (in days) of preimago development of male and female parasitoids and host fly. Data (three parameters) generated on the functional response of female parasitoids to increasing numbers of host eggs (test 3) were analysed with I-way Analysis of Variance (ANOVA) (SPSS, 1993). Where applicable, data were first subjected to Levene's test to check for homo-

geneity of variances (SPSS, 1993). Otherwise, data were transformed before analysis, i.e., percentages to arcsine square root of proportion. Mean separation was tested by Tukey–Kramer Honestly Significant Difference (HSD) method at P = 0.05. Untransformed means (±S.E.) were used in the presentation of results.

Results

Test 1. Host response of parasitoid to olive fruit fly-infested fruits. Gravid *F. arisanus* responded to both *B. oleae* or *C. capitata* eggs that were laid naturally in ripe olive or papaya fruits, respectively (Figure 1). Based on counts of parasitoids that were observed on the fruit, overall mean percentages of females that visited papaya and olives were significantly different (30.2 on papaya, 21.3 on olive).

Student *t*-test (*t*-value = 2.8; P < 0.05). The response exhibited by *F. arisanus* showed an asymptotic pattern with plateaus commencing at 80 min (in papaya) or 110 min (in olives) after initial contact of infested fruits by the parasitoids. The mean number of parasitoids found on papaya (= 41.2 ± 1.7) peaked within 2 h after the start of the test while in olives parasitoids took an additional 30 min before peak number of parasitoids (= 29.8 ± 1.8) was observed.

The proportion of parasitoids that showed a typical egg-laying behaviour was more intense on papaya than on olives (Figure 2). An overall average of 66.8 of females alighted on fruits was noted ovipositing in papaya but only 37.9 of females in olives (*t*-value = 3.5; P < 0.05). The mean number of the ovipositing females peaked at $88.0 \pm 5.4\%$ on papaya while at $65.0 \pm 3.5\%$ on olives. Between the two fruit substrates, the host response displayed by female parasitoids was more aggressive in papaya than in olives. In fact, $53.2 \pm 2.6\%$ of females found on fruits commenced to oviposit in medfly-infested papaya within 50 min after start of the assay while on olives $21.2 \pm 1.7\%$ were noted ovipositing within the same time. An average of 50% of females presents on fruits was observed ovipositing on olives not earlier than 120 min after the start of the experiment. Dissection of exposed fruits allowed us to determine that there was as few as 0.97 ± 0.2 *B. oleae* eggs per hole in olive fruit compared with as many as 8.3 ± 1.8 *C. capitata* eggs per hole in papaya.

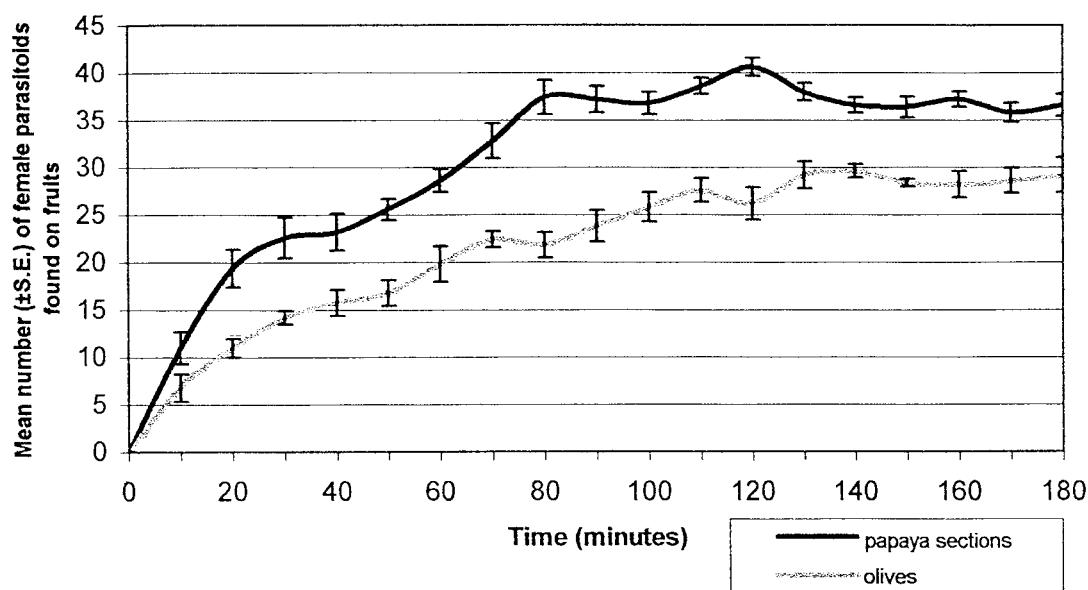*Test 2. Development and pattern of emergence of parasitoid and olive fruit fly.* There was overlap in adult

Figure 1. Mean number of *F. arisanus* females (±S.E.) found on olive or papaya fruits over a 3 h exposition period.

emergence of fruit flies and parasitoids (Figure 3). We recovered 112 *B. oleae* puparia from olives that were exposed to *F. arisanus*. Forty percent of these puparia (*n* = 45) developed into flies and emerged continuously for eight days, that is 26–34 days after olive fruit fly eggs were exposed to parasitoids. Male parasitoids commenced to emerge three days after onset of fly emergence (*n* = 23). Two days later, females eclosed with tail end of emergence completed on the 39th day (*n* = 28). 'Flush' emergence of male and female parasitoids occurred on the 33rd and 36th day, respectively. The preimago development of males (average 33 ± 1.7 days) was two days shorter than that of females (35 ± 1).

Test 3. Effect of host egg number to female parasitoid ratio on olive fruit fly parasitisation. Simultaneous exposure of *B. oleae* eggs to *F. arisanus* at decreasing host egg to female parasitoid ratios corresponded with significant reductions in recovery of host puparia (F = 26.5; df = 45; P < 0.01). Host availability from as few as one to as many as 20 eggs per female parasitoid yielded between 40–10% less puparia than unexposed eggs (control). Apparently, when fewer eggs were made available to the female parasitoids, there were corresponding increases in host mortality (Figure 4). Thus, ratios of 1:20.1:10, 1:5. and 1:1 yielded 70 ± 8, 64 ± 7, 52 ± 10, and 38 ± 14% of recovered puparia, respectively, while in the control treatment we recovered 79 ± 9% of the initially infesting fruits

olive fruit fly eggs. Percent parasitism decreased as the number of host eggs per female increased (Figure 4). Nevertheless, the level of parasitisation obtained in 1:1 host egg to female ratio was significantly higher (82 ± 18%) than what was observed (28–55%) in the other ratios (F = 37.2; df = 36; P < 0.05). Increases in the number of available host eggs per female corresponded with increases in absolute parasitisation. The number of successfully parasitised hosts ranged from 31–380 (Table 1).

Discussion

We found that *B. oleae*-infested olives provided a suitable substrate for oviposition and complete development of *F. arisanus*, although they were not as attractive as the medfly-infested papaya. This may be explained by variation of host egg dispersion and the clutch size in the fruit substrate. In fact, dissection of exposed fruits showed that fewer host eggs were available for oviposition in olives than in papaya. Consequently, more time was spent per fruit by a single female parasitoid searching for *B. oleae* eggs (Holling, 1959). Also, considering that *F. arisanus* had been reared on medfly using papaya as the fruit inoculation substrate (R.C. Bautista, unpubl.; Harris & Okamoto, 1991), it was not surprising that female parasitoids favoured *C. capitata* eggs laid in papaya to *B. oleae* eggs in olives. Moreover, the fact that fruit variety
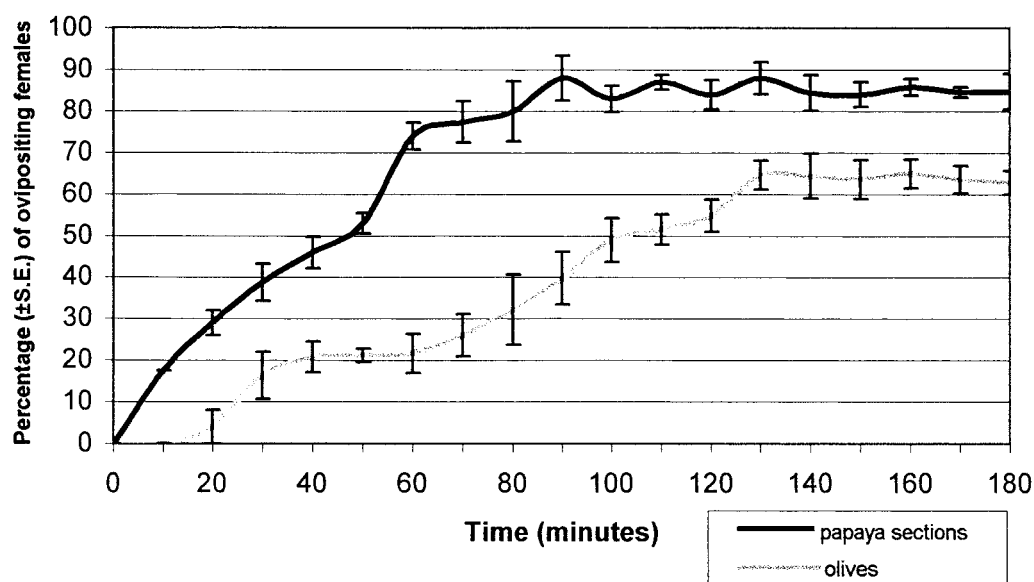
Figure 2. Mean percentage (±S.E.) of *F. arisanus* females found on olive or papaya fruits exhibiting oviposition behaviour over a 3 h exposition period.

Table 1. Bactrocera oleae and *Fopius arisanus* recovery from parasitized (exposed) and unparasitized (control) hosts (actual counts)

	Host egg number to female parasitoid ratio (*n* = olives used in each ratio)				
	1:1 (*n* = 100)	5:1 (*n* = 500)	10:1 (*n* = 1000)	20:1 (*n* = 2000)	Control (*n* = 500)
Recovered pupae	38	260	640	1401	385
Parasitoids	31	145	270	380	
Olive fruit flies	5	110	330	920	365

and fruit odour stimuli could potentially influence the choice made by *F. arisanus* (Bautista & Harris, 1996; Leyva et al., 1991; Messing & Jang, 1992) may have caused differences in our observation.

Our findings nevertheless provided evidence that *F. arisanus* being a polyphagous egg-pupal parasitoid of tephritid fruit flies, has the ability to attack and parasitise a new host (*B. oleae* eggs), infesting an entirely different fruit substrate (olive).

Overlapping patterns in the emergence of host flies (from unparasitised puparia) and male and female parasitoids are similar to those in previous observations (Bautista et al., 1998). However, these authors reported a much shorter developmental time for *F. arisanus* when reared on the oriental fruit fly, *B. dorsalis*. This difference is mostly due to the shorter life cycle of this host reared on an artificial diet (16–23 days at 22–24 °C), than that of *B. oleae* (26–33 days at 20 °C) in our tests fully developed in natural fruits.

Nevertheless, our observation clearly indicated that *F. arisanus* can utilise *B. oleae* as a host, thus sustaining its development and production of progeny. Moreover, levels of parasitism found in our experiments (28–82%) were in the same range as those found for *B. dorsalis*, known as the most suitable host for *F. arisanus* (Harris & Bautista, 1996).

Significant variations of percent development from eggs to puparia were found between exposed and unexposed (control) olive fruit fly eggs. The lowest levels of puparial recovery corresponded to the lowest host egg to parasitoid ratio. Because puparial recovery provide an indirect measure of host fruit fly mortality (Harris & Bautista, 1996), it was speculated that insertion of the egg laying ovipositor by *F. arisanus* exerted a killing effect on the development of the host eggs (Newell & Rathburn, 1951; Haramoto, 1957; Kaya & Nishida, 1968). Host mortality could be interpreted as a negative trait in the *B. oleae-F. arisanus* association,
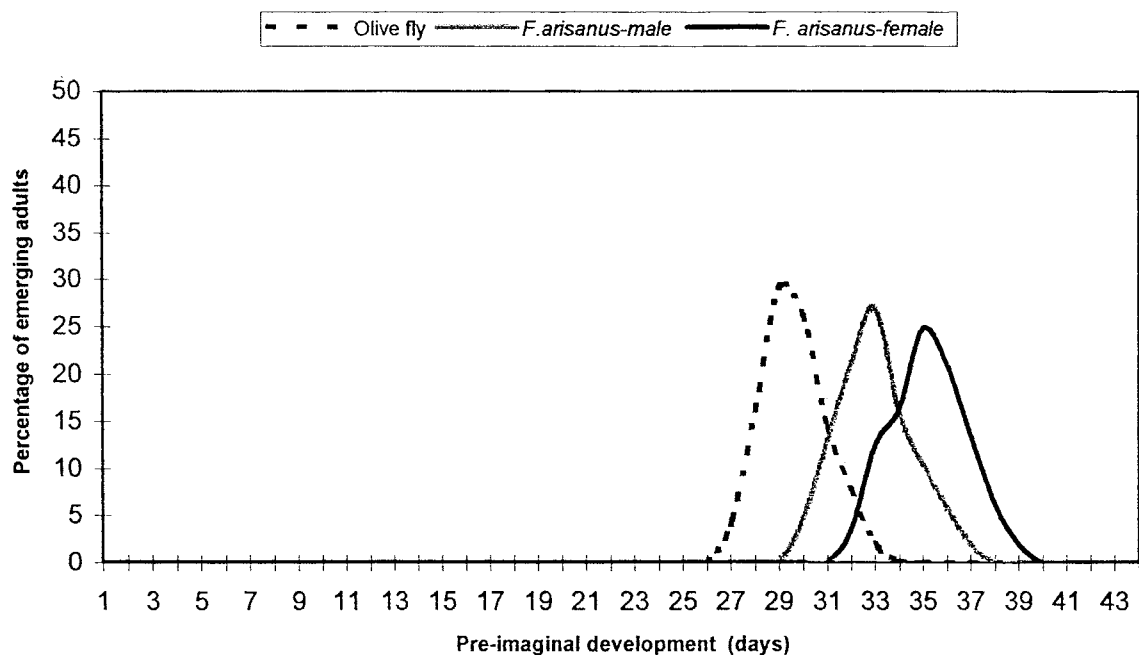
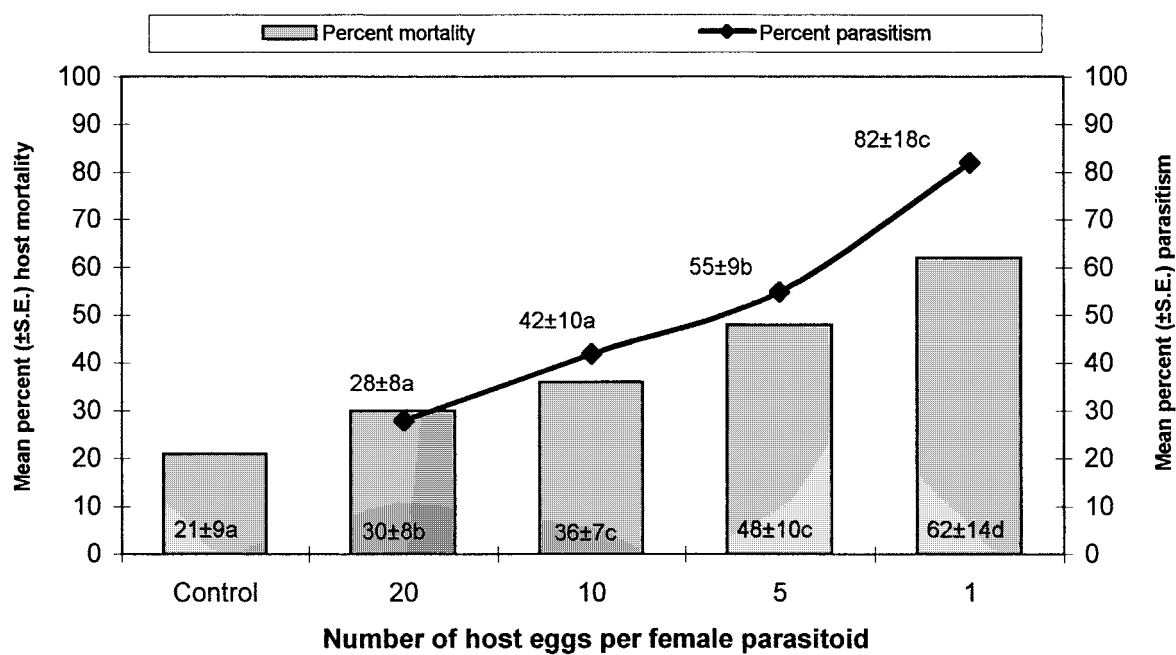Figure 3. Pattern of emergence of *F. arisanus* males and females and *B. oleae*.

Figure 4. Effect of host egg number to female parasitoid ratio on mean percent (±S.E.) host mortality and mean (±S.E.) percent parasitism. Data points with same letter are not significantly different by Tukey's test (P > 0.05).

but actually it is a phenomenon that also occurs when this parasitoid attacks the eggs of its preferred host *B. dorsalis* (Bautista & Harris, 1996).

According to Bautista et al. (1998), the incidence of superparasitised hosts is very low in the laboratory and does not increase in proportion to parasitoid density. This finding may indicate that female *F. arisanus* are able to discriminate unparasitised from previously parasitised hosts (Ramadan et al., 1992; Lawrence et al., 1978). Thus, the killing effect cannot be attributed to superparasitism. As reported by Harris & Bautista (1996), differences in host egg mortality could suggest differences in sensitivity among species to ovipositor insertion of *F. arisanus* during parasitisation.

In our work, parasitisation and the killing effect found on *B. oleae* proved to be parasitoid density dependent, as expected. Nevertheless, a significant host mortality was confirmed (9% less pupae recovered than in control) also at highest host egg availability (20:1), corresponding to a percent parasitism of 28%. Thus, we confirmed that a proportion of killed eggs is always associated with parasitization, even if percent parasitism is low. In addition, the highest host egg availability corresponded to the highest number of successfully parasitised hosts per female *F. arisanus* (Table 1).

Host killing effect and rate of parasitisation have to be taken into consideration as factors in determining the actual effectiveness of a parasitoid against its host populations (Bautista & Harris, 1996). Moreover, according to Kaya & Nishida (1968), with regard to *B. dorsalis* and *C. capitata*, superparasitism is higher in the field where the distribution of fruit fly eggs is patchy compared to the laboratory where females are provided with an ample supply of host eggs.

We expect that all these findings, if confirmed for *B. oleae* in the field, could be of relevant applied value for *B. oleae* biological control. In fact, like other monophagous fruit fly species (such as *Rhagoletis cerasi* L.), this tephritid is inclined to highly disperse its eggs on more fruits, laying only one egg per fruit at early stages of infestation (Cirio, 1971, 1984). Thus, the direct killing of olive fruit fly eggs that results from *F. arisanus* oviposition may make it possible to preserve olive fly oviposited fruits from damage.

Data presented and discussed in this paper represent the preliminary phase of a research project aimed at evaluating the potential of *F. arisanus* as an augmentative biocontrol agent against the olive fruit fly in the Mediterranean Basin. Further research is now in progress with the aim of investigating more thoroughly the functional response of the parasitoid to *B. oleae* (host searching capacity), both in lab and field cage tests, and evaluating its acclimatisation to the Mediterranean climate.

Particular attention will be addressed to the interaction between *F. arisanus* and *C. capitata* in the field, since biological control of the Mediterranean fruit fly by *F. arisanus* augmentative releases has already been successfully carried out in Hawaii (Vargas et al., 1993). Mass rearing of *F. arisanus* on medfly is very simple, nevertheless, as evidenced by our work, this parasitoid maintains the capability to attack olive fruit fly as well. Moreover, considering that *F. arisanus* and *O. concolor* exploit different developmental stages of *B. oleae*, their combined impact on *B. oleae* population dynamics need also be evaluated. Finally, although many scientists generally consider an augmentative biological control strategy among the most benign forms of pest control, the lack of data on the potential non-target effects of introduced opiine parasitoids calls for specific studies before a biological control program can be safely implemented.

References

Baranowski, R., H. Glenn & J. Sivinski, 1993. Biological control of the Caribbean fruit fly (Diptera:Tephritidae). Florida Entomologist 76: 245–251.

Bautista, R. C. & E. J. Harris, 1996. Effect of fruit substrates on paratization of tephritid fruit flies (Diptera) by the parasitoid *Biosteres arisanus* (Hymenoptera: Braconidae). Environmental Entomology 25: 470–475.

Bautista, R. C., E. J. Harris & P. O. Lawrence, 1998. Biology and rearing of the fruit fly parasitoid *Biosteres arisanus*: clues to insectary propagation. Entomologia Experimentalis et Applicata 89: 79–85.

Bautista, R. C., N. Mochizuki, J. P. Spencer, E. J. Harris & D. M. Ichimura, 1999. Mass-rearing of the tephridid fruit fly parasitoid *Fopius arisanus* (Hymenoptera: Braconidae). Biological Control 15: 137–144.

Cirio, U., 1971. Reperti sul meccanismo stimolo-risposta nell'ovideposizione del *Dacus oleae* Gmelin. Redia 52: 577–600.

Cirio, U., 1977. Control of Mediterranean fruit fly by Sterile Insect Technique. Bollettino del Laboratorio di Entomologia Agraria 34: 62–73.

Cirio, U., 1997. Agrichemicals and environmental impact in olive farming. Olivae, 65: 32–39.

Cirio, U. & P. Gherardini, 1984. Egg dispersion and intraspecific larval competition in *Dacus oleae* Gmelin (Diptera Trypetidae). In: 4° Simposio di Dinamica di Popolazioni. Parma, 22–24 Ottobre 1981. Editrice Studium Parmense, Parma, pp. 153–165.

Clausen, C. P., D. W. Clancy & Q. C. Chock, 1965. Biological control of the oriental fruit fly (*Dacus dorsalis* Hendel) and other fruit flies in Hawaii. U.S. Department of Agriculture. Technical Bulletin 1322: 1–102.

Fimiani, P., 1982. Aspetti biologici del *Dacus oleae* (Gmel.) in Campania. C.N.R. Progetto Finalizzato 'Fitofarmaci e fitoregolatori', Sottoprogetto 2: mosche, olive, frutta, ciliegie. Frustula Entomologica 4 (17): 93–97.

Fimiani, P., 1989. Pest status: Mediterranean region. In: A. Robinson & G. Hooper (eds), World Crop Pests, Fruit Flies, Their Biology, Natural Enemies and Control, Vol. 3A. Elsevier, Amsterdam, the Netherlands, pp. 39–50.

Genduso, P., S. Ragusa di Chiara & P. Parenzan, 1994. Allevamento di *Opius concolor* Sz. e sua utilizzazione per il controllo biologico della *Bactrocera oleae* Gmel. In: Innovazione e Prospettive nella Difesa Fitosanitaria. Convegno Ministero delle Risorse Agricole, Alimentari e Forestali, Ferrara 1994. Istituto Sperimentale di Patologia Vegetale, Roma, pp. 213–217.

Haramoto, F. H., 1953. The biology of *Opius oophilus* Fullaway (Braconidae Hymenoptera). M.S. Thesis, University of Hawaii at Manoa, Honolulu, Hawaii.

Haramoto, F. H., 1957. Observations on the biology of *Opius oophilus* Fullaway (Braconidae Hymenoptera). In: Proceeding, 8th Pacific Science Congress of the Pacific Science Assoc., vol. IIIA, Oceanography and Zoology. National Research Council of the Philippines, University of the Philippines, Quezon City, Philippines, pp. 1275–1289.

Harris, E. J. & R. C. Bautista, 1996. Effects of fruit fly host, fruit species, and host egg to female parasitoid ratio on the laboratory rearing of *Biosteres arisanus*. Entomologia Experimentalis et Applicata 79: 187–194.

Harris, E. J. & R. Y. Okamoto, 1991. A method for rearing *Biosteres arisanus* (Hymenoptera: Braconidae) in the laboratory. Journal of Economic Entomology 84: 417–422.

Heim, G., 1984. Effect of insecticidal sprays on predators and indifferent arthropods found on olive trees in the north of Lebanon. In: R. Cavalloro & A. Crovetti (eds), Integrated Pest Control in Olive Groves. A.A. Balkema, Rotterdam, Netherlands, pp. 456–465.

Holling, C. S., 1959. Some characteristics of simple types of predation and parasitism. Canadian Entomologist 91: 385–398.

Kaya, K. H. & T. Nishida, 1968. Superparasitism by *Opius oophilus*, a parasite of the oriental fruit fly. Hawaii Agricultural Experiment station. University of Hawaii, Technical Bulletin No. 73, pp. 31.

Knipling, E. F., 1992. The basic Principles of Insect Population Suppression and Management. U.S. Department of Agriculture Agricultural Handbook No. 512.

Lawrence, P. O., P. D. Greany, J. L. Nation & R. M. Buranowski, 1978. Oviposition behaviour of *Biosteres longicaudatus*, a parasite of the Caribbean fruit fly, *Anastrepha suspensa*. Annals of the Entomological Society of America 71: 253–256.

Leandri, A., V. Pompi, C. Pucci & A. F. Spanedda, 1993. Residues on olives, oil and processing waste water of pesticides used for the control of *Dacus oleae* Gmel. (Diptera: Tephritidae). Anzeiger Scadlingskune, Pflanzenschultz Umweltschutz 66: 48–51.

Leyva, J. L., H. W. Browning & F. E. Gilstrap, 1991. Effect of host fruit species, size and color on paratization of *Anastrepha ludens* (Diptera: Tephritidae) by *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae). Environmental Entomology 20: 1469–1474?

Messing, R. H. & E. B. Jang, 1992. Response of the fruit fly parasitoid *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae) to host-fruit stimuli. Environmental Entomology 21: 1189–1195.

Mitchell, S., N. Tanaka & L. F. Steiner, 1965. Methods of mass culturing melon flies and oriental and Mediterranean fruit flies. USDA, ARS 33–104.

Newell, L. M. & R. Rathbun, 1951. Evaluation of effectiveness of natural enemies of fruit flies. Published by the University of Hawaii. Agricultural Experiment Station. Entomology Department, Quarterly Report, April-June 1951, pp. 12.

Nishida, T. & F. Haramoto, 1953. Immunity of *Dacus cucurbitae* to attack by certain parasites of *Dacus dorsalis*. Journal of Economic Entomology 46: 61–64.

Palacio, I. P., A. G. Ibrahim & R. Ibrahim, 1991. Interspecific competition among opiine parasitoids of the oriental fruit fly, *Bactrocera dorsalis* (Hendel). Philippine Entomologist 8: 1087–1097.

Prokopy, R. J. & R. P. Webster, 1978. Oviposition-deterring pheromone of *Rhagoletis pomonella*, a kairomone for its parasitoid *Opius lectus*. Journal of Chemical Ecology 4: 481–494.

Ramadan, M. M., T. T. Y. Wong & J. W. Beardsley, 1992. Reproductive behaviour of *Biosteres arisanus* (Sonan) (Hymenoptera: Braconidae), an egg-larval parasitoid of the oriental fruit fly, Biological Control 2: 28–34.

SPSS Inc., 1993. Base System User's Guide, Release 6.0. Distributed by Prentice Hall, Chicago, Illinois, USA.

Tzanakakis, M. E., 1989. Small-scale rearing: *Dacus oleae*. In: A. S. Robinson & G. Hooper (eds), World crop Pests, Fruit Flies, Their Biology, Natural Enemies and Control, Vol. 3B. Elsevier, Amsterdam, The Netherlands, pp. 105–118.

Vargas, R. I., J. D. Stark, G. K. Ukida & M. Purcell, 1993. Opiine parasitoids (Hymenoptera: Braconidae) of oriental fruit fly (Diptera: Tephritidae) on Kauai Island, Hawaii: islandwide relative abundance and parasitism rates in wild and orchard Guava habitats. Environmental Entomology 22: 246–253.

Wharton, R. A. & F. E. Gilstrap, 1983. Key to and status of opiinae braconid (Hymenoptera) parasitoids used in biological control of *Ceratitis* and *Dacus s.l.* (Diptera: Tephritidae). Annals of the Entomological Society of America 76: 721–742.

Wharton, R. A., F. E. Gilstrap, R. H. Rhode, M. Fischel-M. & W. G. Hart, 1981. Hymenopterous egg-pupal and larval-pupal parasitoids of *Ceratitis capitata* and *Anastrepha* spp. in Costa Rica. Entomophaga 26: 285–290.

Wong, T. Y. & M. M. Ramadam, 1987. Parasitization of the Mediterranean and oriental fruit flies (Diptera: Tephritidae) in the Kula area of Maui, Hawaii. Journal of Economic Entomology 80: 77–80.